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Ensuring the faithful execution of cytokinesis in *Schizosaccharomyces pombe*

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Abbreviations: SIN, septation initiation network; LatA, Latrunculin A; SPB, spindle pole body; PCB, pombe cell cycle box; PBF, pombe cell cycle box binding factor; CTD, carboxy-terminal domain; CESR, core environmental stress response; GAP, GTPase activating protein; PCH, pombe cdc15 homology; IQGAP, IQ motif and GTPase activating domain; DYRK, dual-specificity tyrosine-regulated kinase

Eukaryotic cells ensure error-free progress through the cell cycle by monitoring 1) the completion of cell cycle events, 2) damage to critical cellular components, or 3) structural changes such as the attachment of kinetochores to the mitotic spindle. In the presence of damage, or in the face of a reduced capacity to complete essential events, cells are capable of delaying the cell cycle so that damage can be repaired, or previous cell cycle phases can proceed to completion. Although such "checkpoints" have been extensively studied in many organisms – and much is understood with respect to the monitoring of DNA replication and DNA damage – little is known with regards to mechanisms that might monitor the completion of cytokinesis. In this review I summarize recent work from the fission yeast, *Schizosaccharomyces pombe*, describing the existence of regulatory modules that aid in ensuring the faithful and reliable execution of cytokinesis. Together, these modules promote the maintenance of a "cytokinesis- competent" state characterized by delayed progression into mitosis and the continuous repair and/or re-establishment of the acto-myosin ring. In this way, fission yeast cells are able to increase the likelihood of successful cell division prior to committing to a subsequent cell cycle. The recent demonstration of conservation between *S. pombe* components of these modules, and human proteins with defined roles in preventing cell division failure, suggest that the lessons learned in *S. pombe* may be applicable to other eukaryotes.

The Fission Yeast, *Schizosaccharomyces pombe*

The fission yeast, *Schizosaccharomyces pombe*, has been used extensively as a model system for the molecular-level understanding of a diverse array of biological processes (e.g., cell cycle control, RNA interference, cytoskeletal dynamics, DNA damage/repair).[1-7] Interestingly, of the ~5000 genes in *S. pombe*, at least 172 share significant similarity to human genes whose mis-regulation leads to disease, and at least 23 share significant similarity to human genes with roles in the development of cancer.[6] Thus, *S. pombe* is an excellent model with which to understand the complex regulation of genetic networks and the consequences of their mis-regulation. Nowhere has this been more apparent than in the study of cytokinesis, where a multitude of recent studies have dramatically increased our knowledge of the mechanisms governing this fundamental process.[5,8-12] In many respects these studies have not only increased our knowledge regarding cytokinesis, but have also increased our general understanding of 1) how eukaryotic cells assemble and regulate complex genetic networks and 2) how these regulatory modules relate to higher order biological phenomenon.

While much is now known regarding the assembly and constriction of the actomyosin ring, our understanding of the mechanisms (if any) monitoring the completion of cytokinesis is lacking. In this review, I first present a brief summary of the regulatory modules required for the proper spatial and temporal regulation of cytokinesis in fission yeast. Next, I present an in-depth account of evidence that supports the existence of genetic networks with roles in promoting the reliable execution of cell division.

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Spatial and Temporal Regulation of Cytokinesis in Fission Yeast

Cytokinesis comprises the period of the cell cycle in which newly segregated chromosomes are irreversibly separated into two independent daughter cells. While the absence of cytokinesis is tolerated under certain specialized circumstances – the development of Drosophila embryos, for example – it is normally essential for the proliferation and differentiation of actively growing cellular populations.[13,14] In addition, recent work has also established that cytokinesis failure has dire consequences with respect to the maintenance of genomic integrity.[15-17]

Cytokinetic Actomyosin Ring Assembly

In fission yeast, just as in more developmentally complex eukaryotes, cytokinesis is achieved through the regulated assembly

and subsequent constriction of a contractile actomyosin ring. The fission yeast actomyosin ring includes two type II myosin heavy chains, Myo2p, and Myp2, together with their associated light chains, Cdc4p (essential light chain) and Rlc1p (regulatory light chain). In addition, the ring includes the IQGAP related protein, Rng2p, the PCH domain protein, Cdc15p, Cdc12p (formin), Cdc8p (tropomyosin), Cdc3p (profilin), as well as the actin filament cross linking proteins, Ain1p (α-actinin), and Fim1p (fimbrin). Mutations in essential actomyosin ring genes (e.g., *cdc3*, *cdc4*, *cdc8*, *cdc12*, *rng2*, and *myo2*) lead to the so-called "*rng*" phenotype; a phenotype characterized by the inability to assemble proper actomyosin rings and the subsequent appearance of elongated and branched cells with multiple nuclei and disorganized septal depositions.[8,11,12,18]

Contractile rings form from pre-cursor "interphase nodes" containing the analin-like protein Mid1p, the kinesin, Klp8, and the Rho family guanine nucleotide exchange factor, Gef2. Mid1p, which exits the nucleus and positions itself at the medial cortex prior to mitosis, acts as a "marker" for the future site of cell division (see below).[19] In addition to Mid1p, a group of regulatory kinases, Cdr1p, Cdr2p, and Wee1p, are also present in interphase nodes. These kinases, together with the DYRK kinase, Pom1p, play a role in co-ordinating ring constriction with entry into mitosis.[8,10,19-24]

Pom1p localizes to the cell tips where it inhibits the Cdr1p and Cdr2p kinases and aids in restricting Mid1p to the medial region of the cell. As newly divided *S. pombe* cells grow in length – and the population of Pom1p molecules moves further from the medial region – this inhibition is relieved, allowing Cdr1p and Cdr2p to negatively regulate the Wee1p kinase through phosphorylation. In the presence of this negative regulation Wee1p is no longer able to inhibit the function of the cyclin dependent kinase, Cdc2p, thereby promoting the G2 to M transition. Thus, this elegant system allows for the proper spatial positioning of the ring, as well as for the co-ordination of ring assembly with entry into mitosis.[25-28]

Upon entry into mitosis the interphase nodes mature into "cytokinesis nodes" by the sequential recruitment of myosin II (along with its light chains), Rng2p, Cdc15p, and Cdc12p. Once recruited, Cdc12p (formin), together with Cdc3p (profilin), promote actin filament polymerization. With the aid of tropomyosin, fimbrin, and α-actinin, the actin filaments condense into an organized, bundled ring. Two non-exclusive models – the "leading cable" model and the "search, capture, pull and release" model – have been proposed to explain the condensation and constriction process.[8,10-12,20-24]

The Septation Initiation Network

Once formed, the timing of ring constriction is controlled by an elaborate signaling pathway known as the septation initiation network (SIN). This network localizes to the spindle pole body (SPB; the centrosome equivalent in yeast) and triggers ring constriction.[9,29,30] In the absence of SIN signaling the actomyosin ring forms upon entry into mitosis, but then disassembles prematurely in late anaphase.[9,18,31-34]

The Spg1p GTPase acts as the "on-switch" of the SIN. Spg1p is negatively regulated by the two component GTPase activating protein (GAP), Cdc16p-Byr4p. Overexpression of Spg1p, or deletion of either Cdc16p or Byr4p, leads to multiple rounds of ring and septum formation without cell separation and intervening nuclear divisions.[9,35-39] When in its GTP bound form Spg1p promotes the recruitment of the Cdc7p protein kinase, which in-turn recruits the Sid1p-Cdc14p protein kinase complex to the SPB.

The scaffold proteins, Sid4p and Cdc11p also play a crucial role in SIN signaling as they function together in the recruitment of SIN components to the SPB. The most downstream component of the SIN is the Sid2p-Mob1p kinase complex whose enzymatic activity peaks during cytokinesis in a SIN dependent manner. This complex localizes to the SPB throughout the cell cycle, but also accumulates at the division site immediately prior to cytokinesis and is thought to transmit the division signal from the SPB to the actomyosin ring triggering its constriction.[9,29,30,33,40-47] In addition to controlling ring constriction, more recent work has suggested that the SIN may also play a role in ring assembly through the recruitment of Cdc15p.[48]

Regulatory Networks Governing the Linkage between Mitosis and Cytokinesis in *S. pombe*

In fission yeast, it is clear that the initiation of cytokinesis is linked to the completion of the preceding mitosis. This has been demonstrated by experiments involving the spindle checkpoint effector, Mad2p, which, when overexpressed, induces a metaphase arrest with increased levels of the cyclin dependent kinase, Cdc2p.[49] Under these conditions septation is normally prevented, however, in backgrounds where the activity of Cdc2p is reduced, cells are competent to form septa despite their failure to exit mitosis.[31]

The mechanism by which this dependency is enforced involves the SIN components Cdc14p and Sid1p. While the over-expression of Mad2p has no effect on the localization of upstream SIN components, it does prevent the localization of Cdc14p and Sid1p to the SPB. Furthermore, when assayed directly, Cdc14p-Sid1p localization to the SPB occurs subsequent to Cdc2p kinase inactivation.[41] These data suggest that the localization of Sid1p-Cdc14p to the SPB, and thus activation of the SIN, is linked to mitotic exit through regulated inactivation of Cdc2p kinase activity.

Regulatory Networks Governing the Faithful Execution of Cytokinesis in *S. pombe*

While the mechanism described above ensures that cytokinesis only begins upon completion of the previous mitosis, it does not ensure that the completion of cytokinesis precedes entry into the subsequent mitosis. The remainder of this review will focus on the mechanisms present in fission yeast which maintain this dependency.

"Group I" vs. "Group II" Cytokinesis Mutants

The existence of a cytokinesis monitoring system was first suggested by experiments in which the nuclear cycle kinetics of temperature sensitive cytokinesis mutants were carefully analyzed.[50-52] These experiments clearly revealed two classes of mutant behavior. While SIN mutants accumulate nuclei at a rate indistinguishable from wild-type cells at the restrictive temperature, *rng* mutants accumulate nuclei at a reduced rate. Furthermore, the delay seen in *rng* mutants is abolished in SIN mutant backgrounds. This indicates that *rng* mutants are being affected by a SIN dependent mechanism capable of delaying cell cycle progression. Cytokinesis mutants can thus be classified into two groups: Group I (comprising mutants that exhibit a delay in nuclear cycle progression upon cytokinetic perturbation), and Group II (comprising the SIN mutants, which proceed with the nuclear cycle unhindered upon failure in cytokinesis).[53]

The Clp1p/Flp1p Phosphatase

The Cdc14p family of phosphatases is comprised of a highly conserved group of cell cycle regulators that function, at least in part, through dephosphorylating Cdc2p substrates.[54] Interestingly, the fission yeast Cdc14p family ortholog, Clp1p/Flp1p, has been strongly implicated as being a key regulator of the cytokinesis monitoring system. First, unlike *rng* single mutants (which are delayed in nuclear cycle progression), *clp1*Δ *rng* double mutants exhibit normal nuclear cycle kinetics upon shift to the restrictive temperature (thus placing the *clp1* gene deletion in the type II mutant category).[50,52] Second, *clp1*Δ mutants maintain a smaller cell size than wild type. This so called "semi-wee" phenotype is displayed by cells in which the transition from G2- to M-phase is accelerated and is a strong indication that Clp1p acts as a negative regulator of Cdc2p in promoting mitotic entry. Third, in contrast to *clp1*Δ and *rng* single mutants – which are able to grow and form colonies at semi-permissive temperatures – *clp1*Δ *rng* double mutants fail in cytokinesis and are inviable under these same growth conditions.[53] Lastly, upon entry into mitosis, a sub- population of Clp1p re-localizes from the nucleolus to the cytokinetic ring (as well as to the cytoplasm and the mitotic spindle).[50,52] Taken together, these data point to Clp1p as being a likely candidate for maintaining the cell cycle delay observed upon perturbation of cell division structures, and show that *clp1*Δ cells are hyper-sensitive to a variety of mutations that disrupt the function of the actomyosin ring. Further evidence extending these observations – and showing that Clp1p confers a definitive survival advantage – has come from experiments utilizing low doses of the actin depolymerizing drug, Latrunculin A (LatA).

LatA is a commonly used drug that acts by sequestering actin monomers.[55] At high concentrations (20–50 μM) this action prevents the re-assembly of monomers into actin filaments and results in the complete abrogation of F-actin structures within 20 min (and ultimately in cell death).[55,56] However, when used at low concentrations (0.2–0.5 μM), the drug can be used as a tool to mildly perturb the ring and activate the cytokinesis monitoring system. For example, *clp1*Δ cells (synchronized in early G2 by centrifugal elutriation) enter the first mitosis and form actomyosin rings with similar kinetics to wild-type upon low dose LatA treatment. However, in contrast to similarly treated wild-type cells – which maintain the integrity of the ring for up to 120 min – *clp1*Δ mutants exhibit ring fragmentation/disassembly within only 30 min. Furthermore, whereas wild-type cells are able to delay mitotic entry (of the second mitosis) until the actomyosin ring slowly constricts, *clp1*Δ cells proceed with the second mitosis (becoming tetra-nucleate) with kinetics indistinguishable from mock treated controls.[53] Moreover, while *clp1*Δ cells are inviable on solid media containing low dose LatA, wild-type cells are capable of growth and colony formation.[53] Thus, consistent with earlier genetic analysis, these data strongly indicate that Clp1p mediates a survival advantage upon LatA treatment through both delaying cell cycle progression and by stabilizing the actomyosin ring.

A Dual Role for Clp1p in Preventing Cytokinesis Failure

An intriguing question that arises when considering the cytokinesis monitoring system concerns the relationship between Clp1p mediated cell cycle arrest and actomyosin ring stability. Clp1p plays a definitive role in regulating the transition from interphase to mitosis. Thus, is increased stability of the actomyosin ring simply the indirect consequence of cell cycle delay, or does Clp1p play a direct role in stabilizing the ring?

To answer this question temperature-sensitive mutations in the mitotic inducer *cdc25* can be used to provide a Clp1p independent cell cycle block upon low dose LatA treatment. Using this strategy it is thus possible to assay actomyosin ring stability under conditions where Clp1p activity is absent, but an interphase arrest is still in effect. Intriguingly, actomyosin rings in *cdc25–22 clp1*Δ cells, unlike *cdc25–22* controls, do indeed fragment in the presence of low doses of LatA.[53] Thus, Clp1p functions not only to delay cell cycle progression, but also to maintain actomyosin ring integrity. These two functions complement one another, providing the cell with a lengthened duration in which to properly constrict the actomyosin ring and successfully complete cytokinesis. It is of particular interest to note the similarity between this bimodal mechanism and other bimodal responses in other characterized checkpoints. For example, in budding yeast, the checkpoint kinase Mec1p is required not only to stabilize slowed or stalled replication forks, but also to delay progress through S phase.[57]

Clp1p Increases the Duration of SIN Signaling

One of the most intriguing aspects of SIN mutants is their ability to form, but not constrict actomyosin rings. Upon shift to the restrictive temperature SIN mutants form rings which are similar to those observed in wild-type cells, however, unlike wild-type cells, these rings disassemble in late anaphase resulting in cytokinesis failure.[58,59] This phenotype is remarkably similar to the phenotype displayed by *clp1*Δ cells upon treatment with low

doses of LatA. Under these conditions *clp1*Δ cells are also competent to form actomyosin rings, but these rings disassemble in a similar fashion to SIN mutants prior to constriction.[53]

To help shed light on the relationship between Clp1p and the SIN, the localization of SIN components (Cdc7p and Sid1p) were examined upon low dose LatA treatment in *clp1*Δ and wild-type backgrounds.[53] Cdc7p and Sid1p localize to a single SPB during late mitosis, and remain there until the completion of cytokinesis. Thus, the localization of these components to the SPB serves as a marker of active SIN signaling.[60]

Interestingly, these studies echoed results obtained when examining actomyosin ring stability. Cdc7p and Sid1p localize to the SPB with similar kinetics in synchronized, LatA treated wild-type and *clp1*Δ cells. However, while wild-type cells are able to maintain Cdc7p and Sid1p to the SPB for prolonged periods of time (up to 240 min), *clp1*Δ cells display a dramatic drop in the percentage of cells with these components at the SPB.[53] In addition, ring fragmentation in low dose LatA treated *clp1*Δ cells can be rescued by mutations resulting in hyper-activation of the SIN.[53] Thus, taking all data together, these results strongly suggest that Clp1p functions to extend the duration of SIN signaling upon cytokinetic perturbations, thereby providing an improved opportunity to properly constrict the ring.

Interestingly, the Clp1p dependent maintenance of SIN activity complements work examining the DNA damage checkpoint in fission yeast. In this work the authors demonstrate that checkpoint arrest in the presence of DNA damage must be actively maintained through the function of the Chk1p protein kinase.[61] This parallels the function of Clp1p in the sense that Clp1p is not required to activate the SIN (*clp1*Δ mutants are fully capable of constricting rings and forming septa under normal growth conditions), but is important in extending the duration of SIN signaling (as needed) in the presence of damage to the cell division machinery.

Clp1p and the SIN function in a Positive Feedback Loop

Like members of the SIN, Clp1p displays cell cycle regulated changes in its sub-cellular localization during normal logarithmic growth. In interphase, Clp1p is present in the nucleolus and SPB. However, upon mitotic entry, Clp1p re-localizes to the cytoplasm/mitotic spindle/actomyosin ring and remains at these locations until the completion of cytokinesis.[50,52]

Intriguingly, upon LatA treatment Clp1p is retained in the cytoplasm for prolonged periods of time. This retention is mediated through the action of the SIN kinase, Sid2p, and the 14–3-3 protein, Rad24p. Upon SIN activation, Sid2p phosphorylates Clp1p creating a binding site for the Rad24p, protein. The interaction between Rad24p and Clp1p thus permits retention of Clp1p in the cytoplasm (**Fig. 1**). As expected, *rad24*Δ cells are unable to maintain prolonged SIN signaling upon LatA treatment leading to inviability and a terminal phenotype indistinguishable from *clp1*Δ cells.[62,63]

Thus, upon cell division stress, SIN signaling is required for the prolonged maintenance of Clp1p in the cytoplasm, and Clp1p is required for the prolonged maintenance of SIN signaling. These

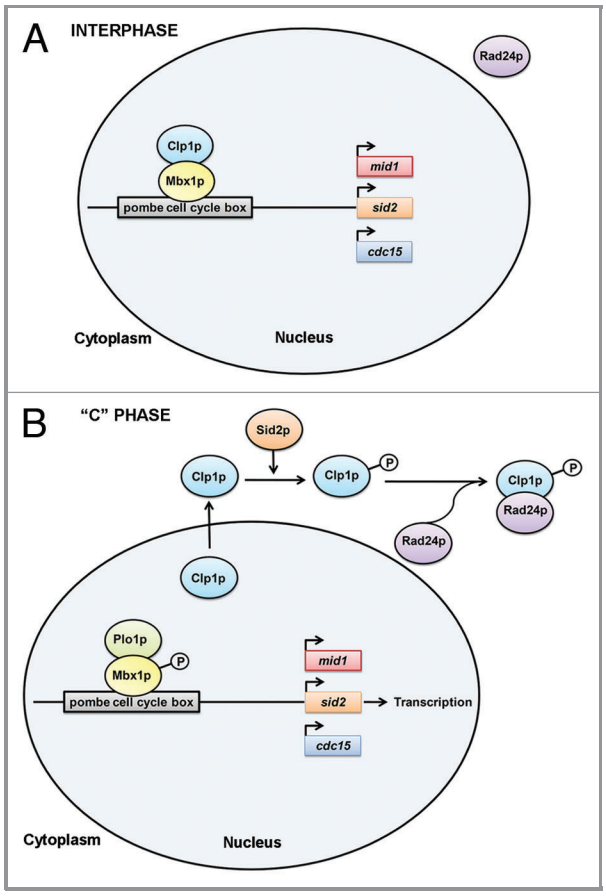

Figure 1. Regulatory dynamics during cytokinesis in *S. pombe*. (A) During interphase, Mbx1p, a component of the pombe cell cycle box binding factor (PBF), is bound and dephosphorylated by Clp1p. In this form Mbx1p aids in repression of the transcription of a set of genes (including *mid1*, *sid2*, and *cdc15*) with roles in cytokinesis. (B) During normal cytokinesis – or upon extension of the cytokinetic (or "C") phase of the cell cycle upon perturbation of the cell division machinery – Plo1p phosphorylates Mbx1p, relieving its inhibitory effect on the transcription of cytokinesis genes such as *sid2*. Sid2p phosphorylates Clp1p, creating a binding site for the 14–3-3 protein, Rad24p. Rad24p binding to Clp1p promotes the cytoplasmic retention of Clp1p, preventing it from de-phosphorylating Mbx1p.

data therefore strongly support a model in which Clp1p and the SIN act in a positive feedback loop (cytoplasmic Clp1p promotes SIN activity and active SIN promotes retention of Clp1p in the cytoplasm). In this way the cytokinetic phase of the cell cycle may be extended and cell cycle progress delayed. Together these mechanisms promote the successful constriction of the actomyosin ring prior to the initiation of mitosis.

Regulation of Gene Expression by Clp1p

Interestingly, Clp1p may also aid in maintaining a "cytokinesis competent" state through affecting cell cycle dependent gene-expression. In *S. pombe*, a trans-acting transcription factor complex (pombe cell cycle box binding factor or PBF) controls a wave of transcription at the M-G1 interval. Not surprisingly, the

genes in this wave encode proteins (including Sid2p and Mid1p) with roles in cytokinesis and cell division. Remarkably, one component of this complex, Mbx1p, is a substrate of Clp1p.[64] When present in the nucleus, Clp1p, binds to and dephosphorylates Mbx1p, thereby aiding in the repression of transcription of the M-G1 wave. In contrast, when Clp1p is present in the cytoplasm, Mbx1p is free to be phosphorylated (by the polo family kinase, Plo1p), thus stimulating transcription of the M-G1 wave.[64,65] Thus, this transcriptional mechanism provides another avenue by which the cytoplasmic retention of Clp1p promotes a "cytokinesis competent" state conducive to the successful completion of cell division (**Fig. 1**).

Genome Wide Screens Identify Novel Regulatory Modules with Roles in Preventing Cytokinesis Failure

As described above, the role of regulators such as Clp1p and Rad24p in monitoring cytokinesis can be revealed experimentally through the treatment of gene deletion mutants with low doses of LatA. Thus, comprehensive and unbiased genome-wide screens – based on hyper-sensitivity to LatA – can be used to identify novel regulatory components of genetic networks with roles in promoting successful cell division. Just such a screen identified the *lsk1* (latrunculin sensitive kinase knockout) gene. Similar to *clp1*Δ cells, *lsk1*Δ mutants appear normal under typical growth conditions, but display severe cytokinesis defects upon perturbation of the cell division machinery. Furthermore – as shown by genetic analysis with various hypo- and hyper-active SIN mutants – Lsk1p acts as a positive regulator of the SIN.[66]

Somewhat surprisingly – given its role in regulating cytokinesis – the *lsk1* gene encodes a kinase that specifically phosphorylates the Ser-2 residues present in the heptad repeats ($Y_1S_2P_3T_4S_5P_6S_7$) of the carboxy terminal domain (CTD) of the largest subunit of RNA polymerase II, Rpb1p.[67] Lsk1p displays significant sequence similarity to human Cdk9p, which, together with cyclin T, forms the p-TEFb complex that targets Ser-2 residues of the RNA pol II CTD and modulates pre-mRNA processing.[67,68] Importantly, *S. pombe* cells bearing *rpb1* alleles (*rpb1–12XS2ACTD*) encoding 12 copies of a mutant heptad in which alanine is substituted for the second serine (in order to mimic the non-phosphorylated state) display cytokinesis phenotypes indistinguishable from *lsk1*Δ strains.[69] These data are consistent with a model in which the Ser-2 residues of the CTD are indeed the biologically relevant targets of Lsk1p with respect to cytokinesis. Furthermore, these data suggest that upstream kinases might act through the CTD to selectively control the transcription of certain gene subsets.

Interestingly, while gene expression profiling of mutants impaired in Ser-2 phosphorylation display little change with respect to the transcription of most genes, a small sub-set of genes affecting cytokinesis – including the actin interacting protein, Aip1p, the Clp1p interacting protein, Nsk1p, and the SPB protein, Cut12p – were found to be differentially regulated.[70] The identification of these genes (particularly Nsk1p, a known interactor of the critical Clp1p phosphatase), together with genetic data showing that Lsk1p is a positive regulator of SIN signaling, suggests a model in which Lsk1p dependent modulation of transcription may also contribute to maintaining a "cytokinesis competent" state.

The final group of regulators to be discussed define components of a putative histone de-acetylase (HDAC) complex. Identified through a comprehensive genome wide screen of gene deletion mutants, Set3p, Snt1p, and Hif2p, are required to prevent cytokinesis failure in response to LatA treatment.[71] Moreover, consistent with a role in responding to LatA induced damage to the cell division machinery, the protein levels of all three regulators increase 2–3 fold upon challenge with the drug.[71]

While global gene expression profiling of *set3*Δ cells revealed that cytokinesis genes were not affected, the analysis did show that Set3p was required to regulate genes with roles in the cellular response to stress. In contrast to wild-type cells, which respond to LatA treatment with strong induction/repression of the core environmental stress response genes (~60% of the CESR genes differentially regulated), *set3*Δ mutants are unable to properly modulate the CESR genes (only 1% of the CESR genes differentially regulated).[71,72] Thus, cytokinetic failure in *set3*Δ cells may be a manifestation of the mutant's inability to properly adapt to the presence of LatA leading to direct and/or indirect effects on the function of the cytokinetic machinery.

When considering this last group of regulators it is particularly intriguing to note that orthologs of Hif2p, Set3p, and Snt1p exist in humans (TBL1X, MLL5, and NCOR2, respectively). Furthermore – as might be expected based on the selection criteria used in the fission yeast screen – these human orthologs have themselves been shown to play a role in cytokinesis in human cells.[73] In this study the researchers discovered that the knockdown of either *MLL5*, *TBL1X*, or *NCOR2* resulted in defects in furrow ingression, subsequent cytokinesis failure, and finally the generation of bi-nucleate (i.e., tetraploid) intermediates with twice the normal number of centrosomes.

Final Thoughts

The importance of understanding the mechanisms utilized by eukaryotes to ensure the dependable execution of cytokinesis was first articulated by Theodor Boveri almost 100 y ago.[74] In his classic work "*Concerning the origin of malignant tumours*" Boveri hypothesized that tetraploid intermediates – derived from either cytokinetic failure or cell fusion – might undergo chaotic multipolar mitoses leading to numerical and/or structural chromosomal defects.

Recent evidence provides experimental support for Boveri's ideas. For instance, tetraploid mouse mammary epithelial cells generated by the inhibition of cytokinesis display increased rates of aneuploidy and (when transplanted into nude mice) give rise to malignant tumors at greater rates than controls.[15-17,75] These results suggest that mechanisms promoting the dependable execution of cytokinesis may be important in maintaining genomic integrity and in preventing carcinogenesis. Thus, in the broadest sense, an understanding of these pathways may provide a better understanding of one "route" by which eukaryotic cells become tumorigenic in multicellular organisms

(for reviews on this subject and a detailed discussion of further experimental evidence please see refs. 15–17).

This last thought raises the question of whether these regulatory modules – which all seem to promote and/or aid in establishing a cellular state conducive to cytokinesis – are specific to *S. pombe*, or whether they may also be of relevance to cytokinesis in more developmentally complex eukaryotes? While highly speculative, some work in mammalian cells suggests that a monitoring system may very well exist in higher eukaryotes. For example cells deficient for phosphotidylethanolamine are unable to complete cytokinesis, and arrest with a stable actomyosin ring and interphase nuclei.[76] Furthermore, mammalian cells treated with a myosin II ATPase inhibitor also arrest with two nuclei and a stable actomyosin ring.[77] In any event, future work will undoubtedly continue to expand our knowledge regarding these regulatory systems and provide further insight into their molecular nature and their relationship to genomic stability.

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